How to improve the dosing of chemicals in high-throughput *in vitro* mammalian cell assays

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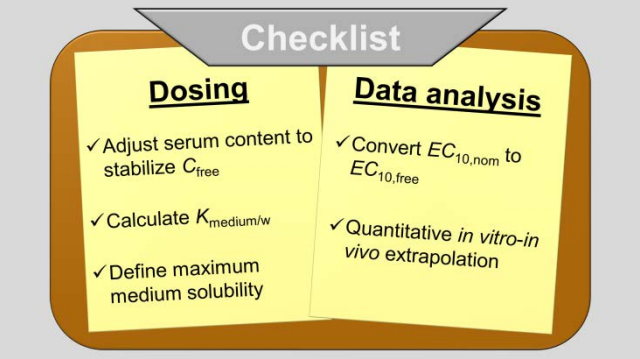

Checklist
Dosing
✓Adjust serum content to stabilize C_{free}
✓Calculate $K_{medium/w}$
✓Define maximum medium solubility
Data analysis
✓Convert $EC_{10,nom}$ to $EC_{10,free}$
✓Quantitative *in vitro-in vivo* extrapolation

Abstract

Controlling the exposure of chemicals in *in vitro* mammalian cell assays is an important prerequisite for the application of *in vitro* methods in risk and hazard assessment of chemicals. Existing models require numerous physicochemical and system parameters to quantify the effective concentration in the assay. Synthesizing these studies, this article briefly communicates how the protein-rich supplement in the medium can be utilized to adjust constant and quantifiable exposure concentrations, without the need for measurements and complex modelling. We present a simplified mass balance equation based on chemical properties and system parameters from openly accessible databases, which can be used to adjust the dose of chemicals in the exposure medium leading to defined and stable freely dissolved concentrations (C_{free}). The proposed framework prevents experimental artefacts associated with the use of co-solvents and medium oversaturation and enables the conversion of *in vitro* effect data to freely dissolved effect concentrations (EC_{free}), which can directly be applied in quantitative *in vitro* to *in vivo* extrapolation models and compared to other exposure scenarios.

1. Introduction

In vitro mammalian cell assays are increasingly applied in risk and hazard assessment of chemicals and their implementation in high-throughput screening (HTS) format helps to meet the high demands on effect data for the increasing number and variety of anthropogenic chemicals. The successful implementation of *in vitro* cell assays in the routine effect characterization of chemicals requires robust and reliable dosing strategies that are applicable in HTS formats. Here, the dosing of chemicals *in vitro* refers to both the entire process of transferring chemicals to the assay medium and the consideration of the resulting effective concentration triggering the effect.

The application of *in vitro* cell assays in high tier multiwell plates (e.g., 384- and 1536-well plates) coupled with the use of robotic pipetting systems allows the simultaneous effect characterization of thousands of chemicals. One of the biggest efforts hitherto is the implementation of the "Toxicology in the 21st Century" (Tox21) program, an HTS platform in which 10,000 chemicals were screened in 70 *in vitro* test systems with cells and isolated subcellular biomarkers (Tox21 10K library, U.S. EPA). The implementation of *in vitro* test systems on such small scale complicates the analytical measurement of exposure concentrations. In toxicology, observed effects are commonly linked to an administered dose (amount of chemical per unit bodyweight), whereas for *in vitro* assays, the nominal concentration (C_{nom}), which is the added amount of chemical per volume bioassay, is used as the typical exposure metric.[1-3] Both the dose *in vivo* and C_{nom} *in vitro* are in most cases not representative for the toxicological effective concentration as the chemicals partition between different compartments of the system.[4] The use of C_{nom} can obscure whether differences between *in vitro* platforms are truly the result of intrinsic differences in sensitivity between the applied cell lines, the receptor expression and/or reporter gene design, or whether they were simply caused by differences in exposure. Because of the lack of exposure

control, *in vitro* assays were criticised for their reduced sensitivity, low inter-assay comparability, and the limited suitability to predict effects on the *in vivo* level.[5] These apparent disadvantages can be overcome by better understanding the dosing process and the partitioning of chemicals in *in vitro* systems.

For better comparability and interpretability of *in vitro* effect data, the freely dissolved concentration (C_{free}) was proposed as quantitative metric because it is considered the chemical concentration that is available for passive uptake by diffusion through membranes.[6] It has been shown that equal C_{free} under different exposure scenarios result in equal effects.[7,8] A critical prerequisite for the successful application of *in vitro* cell assays beyond qualitative effect fingerprinting is their ability to quantitatively predict effects on the whole-organism level, referred to as quantitative *in vitro* to *in vivo* extrapolation (QIVIVE). In QIVIVE models, C_{nom} in the *in vitro* exposure medium is typically directly related to the steady-state plasma concentration *in vivo*,[9,10] but these QIVIVE models can potentially be improved by comparing C_{free} *in vitro* with C_{free} in human plasma,[11] as explained later in the manuscript.

Because of the miniaturization of the test systems, experimental quantification and adjustment of C_{free} are difficult to integrate into the routine application of *in vitro* cell assays in HTS. Therefore, *in vitro* exposure models were developed to quantify C_{free}.[12-14] The common basis of these models is the application of chemical partition constants to quantify the chemical fate in the assay system. The partition constant *K* of a chemical is the concentration ratio of the chemical in two phases at equilibrium.

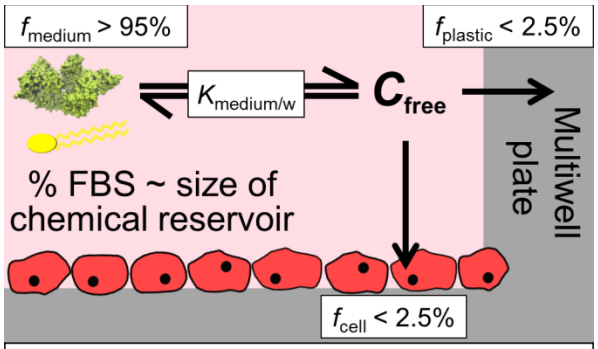
$$K_{1/2} = \frac{C_1}{C_2} \quad (1)$$

Mass balance models describing *in vitro* assays assume instantaneous chemical equilibrium between the different sorptive phases. These models are simplified by the general assumption that proteins and lipids are the sorptive phases in the medium and cells and can be surrogated by generic biomolecules like bovine serum albumin (BSA) and phospholipid liposomes (lip). There are also more comprehensive models that include partitioning kinetics of chemicals.[13,15] The application of these more recent models demands advanced mathematical and physicochemical knowledge, which limits their routine adoption in bioassay laboratories. However, the model results and experiments in earlier studies indicated that this high complexity is not needed to significantly improve the routine dosimetry in HTS *in vitro* assays. Here, we delineate how the knowledge on partitioning of chemicals in standardized *in vitro* cell assays can be condensed to a single simplified mass balance equation that can be applied to increase the quality of dosing in *in vitro* cell assays without the need of complex models and laborious analytical methods. This article focuses on *in vitro* cell assays in HTS platforms that apply commercial multiwell plates covered with a plastic lid or sealed with a breathable foil under the plastic lid, hence essentially a system open to air. Testing volatile chemicals in these test systems is not feasible because of continuous chemical losses by volatilization, therefore, the following statements are only valid for chemicals with an air-water partitioning constant that is smaller than approximately 4×10^{-5}.[16]

2. Proposed framework

2.1 How to use the foetal bovine serum to control and stabilize C_{free}

Synthesizing earlier studies, this section demonstrates how the seemingly complex multicompartment *in vitro* test system can be simplified by adjusting the content of the foetal bovine serum (FBS) in the medium, yielding non-depletive, stable exposure conditions. FBS is commonly applied as nutrient supply for *in vitro* mammalian cells to ensure optimal cell health and growth during the incubation period. FBS has a high protein content and contains important micronutrients like vitamins, salts, and growth hormones.[17] Binding of chemicals to FBS proteins was early recognized to reduce C_{free} and hence the apparent sensitivity of the assay.[18,19] Our recent model study on partitioning of neutral and ionisable chemicals in the Tox21 reporter gene assays confirmed that the medium FBS is the dominant sorptive sink in the assay system, with molar fractions of any chemical in the medium of > 84%.[14] The extent of chemical partitioning to the medium increased with increasing medium volume and FBS content, which ranged between 0.5% and 10% FBS in the Tox21 reporter gene assays. The extensive binding to medium components leads to lower concentrations in the cells, but the large reservoir of reversibly bound chemicals can compensate for any other loss processes, leading to stable freely dissolved concentrations in the exposure medium (Figure 1), which requires rapid desorption from the FBS, which can be assumed as desorption kinetics from BSA were measured to be in range of minutes even for very hydrophobic chemicals.[20] Due to the analogy to conventional passive dosing, in which chemical exposure in the medium is kept constant by desorption from a chemical-saturated polymer, we introduced the term serum-mediated passive dosing (SMPD).[21]



Standard setup of HTS *in vitro* cell assays			
Plate format	96-well	384-well	1536-well
Medium volume	120 µL	40 µL	6 µL
Cell number	10,000	5,000	2,000
Required % FBS	≥ 3%	≥ 5%	≥ 10%

Figure 1 The freely dissolved concentration in the exposure medium (C_{free}) is depleted by cellular uptake and diffusion into the multiwell plate. The required medium FBS contents that reduce the depletion of C_{free} to < 5% over the 24-h assay duration are reported for common, representative assay setups. Calculations were based on experimentally parameterized models,[14,15] which can be adopted to perform own calculations for varying cell numbers, medium volumes, FBS contents, and multiwell plates.

Ensuring stable exposure concentrations in the medium by SMPD requires ≥ 3% FBS content in 96-well plates, ≥ 5% FBS content in 384-well plates and ≥ 10% FBS content in 1536-well plates (Figure 1). The values were predicted based on the experimentally parameterized mass balance and kinetic models.[14,15] Note that for chemicals with a sorption affinity to lipids over two magnitudes higher than their sorption affinity to proteins, SMPD using the suggested FBS contents can be limited. Generally, an increased size of the chemical reservoir favors the applicability of SMPD, whereas a combination of a low FBS content (0.5%) with a small medium volume (6 µL) in 1536-well plates can ultimately lead to a depletion of the FBS reservoir by > 20%. Provided that the FBS content is sufficiently high or the volume of the medium is sufficiently large to keep the chemical exposure in the medium constant, the dosing of chemicals in *in vitro* cell assays can be simulated by a parsimonious model that only requires few input data. In order to maintain stable chemical concentrations in the exposure medium, the SMPD reservoir needs to compensate the

chemical losses by cell uptake, diffusion into multiwell plate materials, and other processes, preferably leading to a chemical depletion of < 5% during the exposure time. Kinetics do not need to be considered in a first approximation because uptake kinetics in cells are relatively fast, as recently shown for ten neutral and ionisable chemicals using a fluorescence microscope and automated image analysis.[21] The medium concentrations of the chemicals remained constant throughout the 24-h assay duration,[21] foremost when applying higher medium FBS concentrations of 5% and 10%. An area under the curve analysis showed that the cellular uptake kinetics reduced the cell exposure by < 20% over the 24 h compared to the exposure that would result from instant cell-medium equilibration. More importantly, increasing the medium FBS content from 0.5% to 10% accelerated the cell-medium equilibration substantially, such as from 3.9 h to 40 min for benzo(a)pyrene.

The diffusion of chemicals into the polystyrene of multiwell plates can significantly and continuously deplete the concentration of chemicals in the colloid-free aqueous exposure medium of *in vivo* assays.[22,23] Our kinetic model study based on experimental polystyrene partition constants and diffusion coefficients evaluated the influence of multiwell plate sorption on the chemical concentrations in the exposure medium of *in vitro* and *in vivo* assays.[15] Even though the polystyrene walls in standardized multiwell plates are thick (up to 1 mm), the effective diffusion depth of the chemicals during the 24-h assay duration was in the range of 20 µm because of the low chemical diffusion coefficients in polystyrene (1.25 to 8.0 x 10^{-16} m^2 s^{-1}). Experiments demonstrated that for hydrophobic chemicals exhibiting high sorptive affinity to polystyrene, the chemical concentrations in the FBS-free exposure medium of *in vivo* assays were significantly reduced.[15,22,23] Contrarily, the depletion in *in vitro* assays was minor because of the high sorptive capacity of the FBS proteins and lipids.[15] The depletion of the exposure medium was lower when

increasing the medium FBS content from 0.5% to 10% because of the increased sorptive capacity of the SMPD reservoir in the medium and the decreased polystyrene-medium partition constant, favoring again the application of high FBS contents in the medium to stabilize exposure.

In previous experimental studies[15,21] it was demonstrated that the total medium concentrations remained stable at sufficiently high FBS contents during the entire exposure period. C_{free} was not significantly reduced in *in vitro* fish and mouse assays that applied 2% and 5% FBS in 24-well plates, and the chemical losses were negligible at higher FBS.[8] Recently, we measured C_{free} directly after dosing and after 24 hours of incubation in two human reporter gene assays that applied 10% and 2% FBS in 96-well plates (AREc32 and PPARγ-GeneBLAzer, respectively).[24] C_{free} of the nine evaluated chemicals remained constant in the PPARγ-GeneBLAzer assay. In AREc32, C_{free} was reduced for two chemicals, which was attributed to cellular metabolism. These experiments verified the suitability of SMPD to stabilize the C_{free} in the medium. Higher medium FBS contents were shown to increase the capacity of the SMPD reservoir and to decrease the impact of cellular uptake kinetics on the attainment of the chemical equilibrium in the cells.[21]

2.2 Key parameters to control exposure

Provided that f_{medium} > 95% (Figure 1), $C_{nom} \approx C_{medium}$ and the maximum concentration of a chemical that can be dosed to the medium of a cell assay, i.e. the medium solubility (S_{medium}), can be calculated by multiplying the water solubility (S_{water}) with the medium-water partition constant ($K_{medium/w}$) (eq. 2). The $K_{medium/w}$ is the partition constant between water and all components of the medium (lipids, proteins, water) and can be measured[25] or predicted as described in Section 2.3.

$$S_{\mathrm{medium}} = S_{\mathrm{water}} \cdot K_{\mathrm{medium/w}} \quad (2)$$

The S_{medium} accounts for the increased solubility of the chemical in the exposure medium resulting from the presence of the FBS proteins and lipids. Experimental and predicted values for S_{water} are available from the Comptox Chemical Dashboard (https://comptox.epa.gov/dashboard).

The freely dissolved effect concentration (EC_{free}) can be estimated from the nominal effect concentration (EC_{nom}) by dividing it by $K_{medium/w}$ (eq. 3).

$$EC_{free} = \frac{EC_{nom}}{K_{medium/w}} \quad (3)$$

Hence, with one parameter ($K_{medium/w}$) we can greatly improve the dosing of chemicals and the interpretation of *in vitro* data (Figure 2).

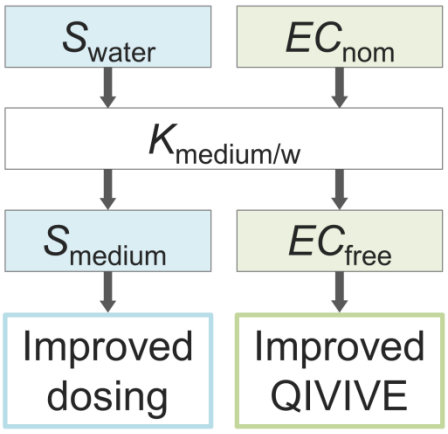


Figure 2 Simplified framework that applies $K_{medium/w}$ to improve the dosing of chemicals in the exposure medium by adjusting S_{medium} and to enable the quantitative extrapolation of *in vitro* effect data to *in vivo* exposure scenarios by conversion of nominal (EC_{nom}) to freely dissolved effect concentrations (EC_{free}).

2.3 Prediction of medium-water partitioning

$K_{medium/w}$ can be predicted from a mass balance model and the lipid- and protein-water partition constants (eq. 4). The $K_{medium/w}$ depends on the phase volume fractions of sorptive proteins, lipids and the non-sorptive aqueous phase in the medium, for which experimental data were reported:[14] 4.6% protein, 0.15% lipid and 95.25% water or non-sorptive phases for different types of commercially available FBS. The fraction of FBS complemented to the medium is described by

β_{FBS}. BSA and liposomes can be used as surrogates for the FBS proteins and lipids in the exposure medium, and the chemical partitioning can be quantified by the BSA-water and liposome-water partition constants ($K_{\mathrm{BSA/w}}$ and $K_{\mathrm{lip/w}}$).

$$K_{\mathrm{medium/w}} = 0.046 \cdot \beta_{\mathrm{FBS}} \cdot K_{\mathrm{BSA/w}} + 0.0015 \cdot \beta_{\mathrm{FBS}} \cdot K_{\mathrm{lip/w}} + 0.9525 \cdot \beta_{\mathrm{FBS}} + (1 - \beta_{\mathrm{FBS}}) \quad (4)$$

For the prediction of both, $K_{\mathrm{BSA/w}}$ (eq. 5) and $K_{\mathrm{lip/w}}$ (eq. 6) for neutral organic chemicals, one can use experimentally parameterized quantitative structure-activity relationships (QSARs) based on the chemicals' octanol-water partition constant (log K_{ow}).[26,27]

$$\log K_{\mathrm{BSA/w}} = 0.71 \cdot \log K_{\mathrm{ow}} + 0.42 \quad (5)$$

$$\log K_{\mathrm{lip/w}} = 1.01 \cdot \log K_{\mathrm{ow}} + 0.12 \quad (6)$$

Experimental $K_{\mathrm{BSA/w}}$ and $K_{\mathrm{lip/w}}$ as well as more complex poly-parameter linear free energy relationships can also be used to obtain these parameters[26,27] but models based on log K_{ow} are most simple due to the easy accessibility of log K_{ow}, which can be downloaded from the Comptox Chemical Dashboard and is contained in most databases for physicochemical properties.

One can include ionisable chemicals into the framework by replacing the $K_{\mathrm{BSA/w}}$ and $K_{\mathrm{lip/w}}$ by the pH-dependent distribution ratios $D_{\mathrm{BSA/w}}$ (pH 7.4) and $D_{\mathrm{lip/w}}$ (pH 7.4) in eq. 4. These distribution ratios account for the neutral and ionized fraction of the ionisable chemical and their partition constants. Eqs. 5 and 6 are only applicable to the neutral species of ionisable chemicals and simple QSARs for the prediction of $D_{\mathrm{BSA/w}}$ (pH 7.4) and $D_{\mathrm{lip/w}}$ (pH 7.4) have so far not been derived, but there is a continuously growing database of experimental data and more complex prediction models for widely used ionisable chemicals like pharmaceuticals and pesticides.[28,29]

2.4 Recommendations for dosing

The maximum dose in an *in vitro* assay must be below water solubility to avoid precipitations, but it should not be too far below S_{water} to avoid false negative results. We propose to choose S_{medium} to ensure that the maximum effects are included in the concentration-effect curve. In the Tox21 reporter gene assays, all chemicals were dosed at a constant C_{nom} range, hence chemicals were in part dosed above their S_{medium}, like the very hydrophobic benzo(b)fluoranthene and benzo(k)fluoranthene, for which the reported 50% effect concentrations (EC_{50}) were one order of magnitude higher than their S_{medium}.[14] The precipitation of chemicals can lead to an underestimation of the chemicals' potency as C_{free} is overestimated. On the other hand, precipitation can result in a direct contact between the solid chemical and the cells, which would result in a locally higher exposure. More hydrophilic chemicals like genistein were dosed three orders of magnitude below their S_{medium}, so that potential effects at higher concentrations were not captured. If the exposure concentrations are not properly adjusted, the measured *in vitro* effect data cannot be correctly interpreted, therefore it is imperative to account for the S_{medium} of the chemicals before dosing.

To ensure complete dissolution of the test chemicals in the medium, we recommend to transfer the chemical(s) into a larger volume of medium in a separate vial (dosing vial), if necessary by using a solubility agent like dimethyl sulfoxide or methanol. A volatile solvent can be blown down in the vial using nitrogen followed by resolubilisation in the medium. Hydrophobic chemicals are recommended to be pipetted as solvent stock directly into the medium in the dosing vial. By applying this intermediate step, experimental artifacts by solvent droplets that can occur by direct dosing of solvent stocks into the wells are prevented.[30] The final concentration of the solvent in the exposure medium must be lower than 0.1% to prevent solvent-associated effects in the cells. Still, a solvent blank is recommended to evaluate potential effects of the procedure as a whole.

After transferring the chemicals, the dosing vial needs to be vortexed followed by a short equilibration time as the chemical equilibrium between the medium constituents is expected to be attained after up to 50 min for very hydrophobic chemicals, based on the measured sorption kinetics of diverse chemicals to BSA in aqueous medium.[20] To transfer the dosed medium to the assay plate, a dosing plate can be interposed. In order to maintain the maximum S_{medium} for the highest test concentration, a complete exchange of the medium in the exposure well is needed, provided that the cells tolerate the short absence of medium and the removal of the medium is feasible. Alternatively, a partial exchange of the medium can be performed to constantly keep the cells in medium during the procedure.

After incubating the cells in the exposure medium, such as for 24 h in the Tox21 reporter gene assays, the endpoints can be assessed. We recommend to quantify both the cytotoxicity and the specific endpoint of interest (e.g., induction of a reporter gene). The cytotoxicity of a chemical can be expressed as the 10% inhibitory concentration (IC_{10}), at which the cell viability is reduced to 90%. The specific effects can be expressed as 10% effect concentration (EC_{10}). The derivation of 10% effect concentrations from the lower, linear part of concentration-response curves facilitates the discrimination of specific modes of action from the cytotoxic effects.[31] The specificity ratio of a chemical (SR) is the ratio of IC_{10} and EC_{10}, and can be used as measure for the potency of a chemical to exhibit the evaluated specific effect compared to its potential to induce non-specific cytotoxicity.[32] A SR of > 10 indicates a specific effect that is not influenced by cytotoxicity. The derivation of SR does not necessarily require the consideration of C_{free} ($IC_{10,free}$ and $EC_{10,free}$), because it compares different concentrations of the same chemical in one assay.

2.5 Recommendations for QIVIVE

In most *in vitro* databases, the effect concentrations are reported as EC_{nom}, which are calculated as the amount of chemical per volume medium, hence the total medium concentration (C_{medium}). Provided that C_{medium} is kept constant by SMPD, the C_{free} can then be calculated by dividing C_{nom} with $K_{medium/w}$ (eq. 3), yielding the conversion of $IC_{10,nom}$ and $EC_{10,nom}$ into $IC_{10,free}$ and $EC_{10,free}$. Because it can be expected that similar C_{free} in different exposure scenarios result in similar effects, the measured $IC_{10,free}$ and $EC_{10,free}$ can be compared between different chemicals, between *in vitro* assays and to *in vivo* assays to assess differences in the chemicals' potency.

QIVIVE models aim to extrapolate the effects that were measured *in vitro* to the steady-state concentration in human plasma to predict adverse human health effects that result from the given exposure. The application of C_{free} as exposure metric increases the suitability of *in vitro* effect data for QIVIVE approaches as differences in the partitioning between different chemicals and test systems cancel out, as well as differences between C_{free} in the assay and plasma. The $IC_{10,free}$ and $EC_{10,free}$ calculated by eq. 3 can directly be linked to the steady-state unbound concentration in human plasma to assess the likeliness whether the measured *in vitro* effects will be triggered *in vivo*. We emphasize that the calculation of C_{free} by the presented simplified framework is only valid if SMPD leads to constant exposure conditions in the medium (f_{medium} > 95%). Comprehensive mass balance and kinetics models that account for all physicochemical and biological processes in the test system should be preferred over the simplified approach in order to refine C_{free} for the extrapolation. Still, the presented framework can be used to assess whether the measured $IC_{10,free}$ and $EC_{10,free}$ are in the range of human exposure concentrations, which would indicate a risk necessitating further evaluation of the chemical.

2.6 Limitations and challenges

Eq. 4 works robustly for neutral chemicals, but it may result in highly uncertain values for ionisable chemicals and should only be applied for rough estimates for multispecies organic chemicals. As complex models such as 3D-QSARs and COSMOmic for the prediction of $K_{BSA/w}$ and $K_{lip/w}$ for ions are not openly available and require comprehensive training, simple and experimentally-based QSARs and prediction models are needed for the derivation of robust partition constants for ionisable chemicals to increase the reliability of eq. 4 for those chemicals. Despite these limitations, the proposed approach is pragmatic and practical until more measured medium-water partition constants become available.

The applicability of the presented framework is limited to non-volatile and stable chemicals. Rapid degradation of chemicals by abiotic processes (photolysis, hydrolysis) or by cellular metabolism can lead to a substantial depletion of the medium. The testing of very volatile chemicals requires closed systems, which have already been developed but are so far not applicable to HTS.[33] SMPD is generally not applicable to *in vitro* assays that apply media with no or very low nutrient content, as the required amount of sorptive colloids in the medium is not present. The proposed FBS contents suitable for SMPD (Figure 1) were derived for 24-h exposure duration, which is the routine exposure time for reporter gene assays. For longer exposure duration in more complex *in vitro* assays, e.g. 3D assays, continuous depletion by volatilization and well plastic diffusion can eventually reduce C_{free}. Note that FBS is a natural product with variable protein and lipid concentrations between different lots, therefore it is advisable to measure protein and lipid concentrations of each fresh FBS lot to prevent systematic errors when applying eq. 4. Still, the average protein and lipid concentrations for different types of FBS applied in eq. 4 are sufficient to considerably improve the dosing of chemicals. The concept of SMPD relies on high FBS contents in the medium (Figure 1). This increasing use of FBS conflicts with current approaches

to reduce the use of animal products for *in vitro* cell culture.[34] We support the development of animal component-free, chemically defined media (FCS-free database, https://fcs-free.org/), however, their suitability to stabilize exposure needs to be evaluated to assure that defined exposure can be achieved. Macromolecules that can be added at defined concentrations to stabilize C_{free} and C_{cell} can be proteins, lipids, lipoproteins or mixtures of these. In 1536-well plates, the percent by volume concentration of sorptive colloids present in the medium needs to be >0.5% to ensure stable C_{free}. Finally, to ensure that high quality data are obtained through *in vitro* testing, we encourage the development of standardized testing guidelines and data analysis protocols.

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Biographies

Fabian C. Fischer has a background in environmental toxicology and is currently a Postdoc in the Department Cell Toxicology at the UFZ Leipzig, Germany. His research focuses on improving the quality and interpretation of chemical effect assessment using miniaturized bioassays in an high-throughput environment. He develops experimentally parameterized exposure models that predict bioavailable concentrations in different *in vitro* and *in vivo* test systems, driven by the motivation to improve inter-assay comparability and the extrapolation to realistic exposure scenarios in organisms.

Luise Henneberger is working as postdoctoral researcher in the Department of Cell Toxicology at UFZ in an industry-funded research project that improves the quantitative exposure assessment in HTS cell-based bioassays and develops and implements methods for quantitative *in vitro*-to-*in vivo* extrapolation (QIVIVE). After finishing her diploma in food chemistry at the Martin Luther University Halle-Wittenberg, she was working as a research assistant and later as a PhD student at the UFZ in the Department of Analytical Environmental Chemistry. Her dissertation focused on the equilibrium sorption of ionogenic organic chemicals (IOCs) to selected proteins, like serum albumin and muscle protein.

Rita Schlichting is the leader of the HTS/bioassay group in the Department of Cell Toxicology at UFZ. Her PhD focused on *Arabidopsis thaliana*. Afterwards she took up a position at a small biotech company establishing workflows for targeted quantitation of protein silencing in response to RNAi based on complex cellular lysates. Subsequently she worked at Cenix BioScience as a project manager for industry and academic projects based on high-throughput bioanalysis for more than five years. Thus, she has experience in automated liquid handling, the analysis of large data sets as well as the presentation of project results on diverse topics.

Beate I. Escher is professor of Environmental Toxicology at the Eberhard Karls University Tübingen, Germany, and Head of the Department Cell Toxicology at UFZ Leipzig, Germany. Escher has been working on developing scientifically sound assessment tools and methodologies for risk assessment of micropollutants in the environment and in people. She is interested in improving dosing and interpretation of high-throughput *in vitro* bioassays and runs the robotic bioassay platform CITEPro at UFZ (www.ufz.de/citepro). More practical-oriented aspects of her research include passive sampling of sediment and biota and effect-based methods for water quality assessment.